Retroviruses as Gene Therapy Vectors - Promise and Problems

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Overview of Today's Presentation

- Retrovirus Vectors
- SCID-X1 Gene Therapy
- New Approaches – New Questions

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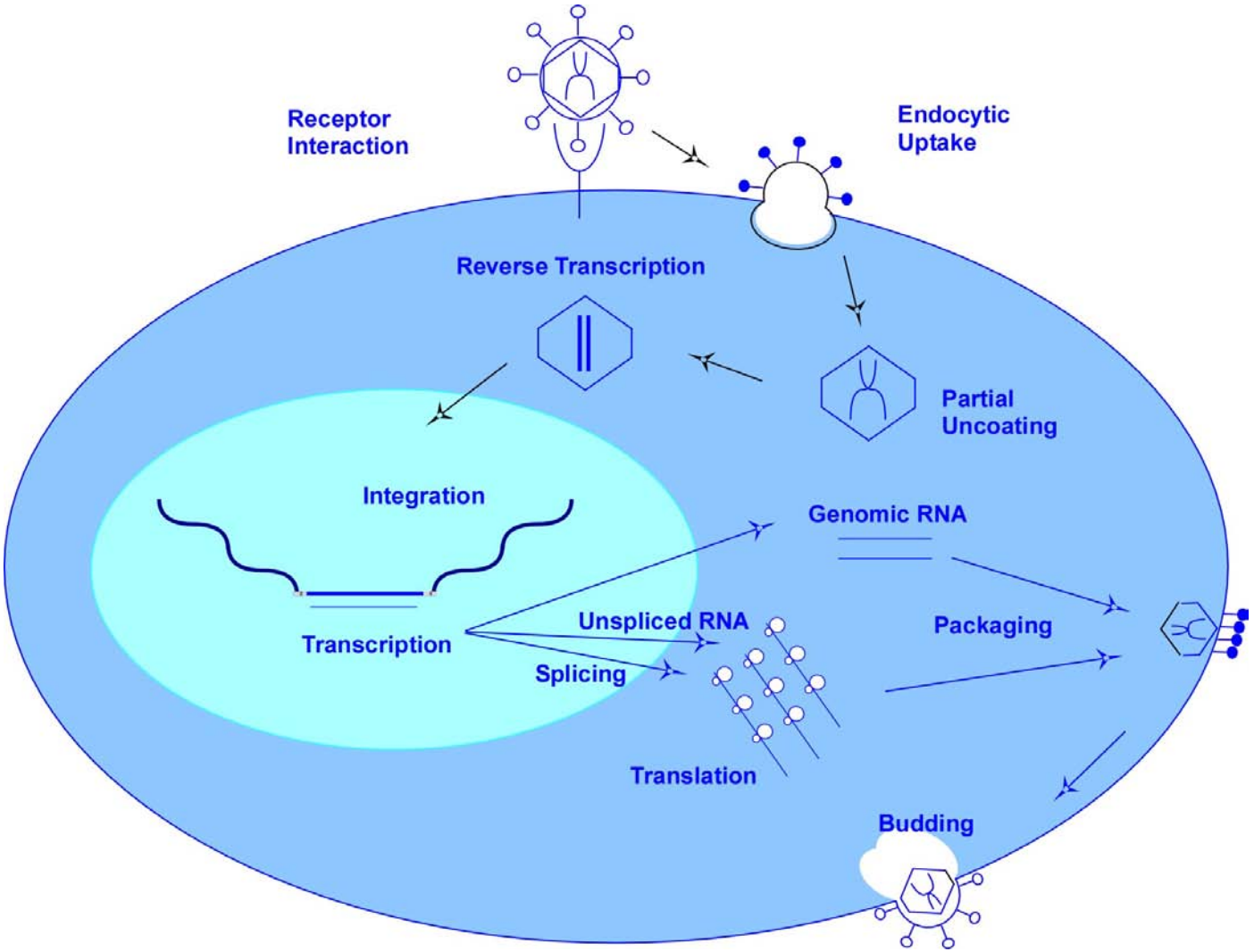
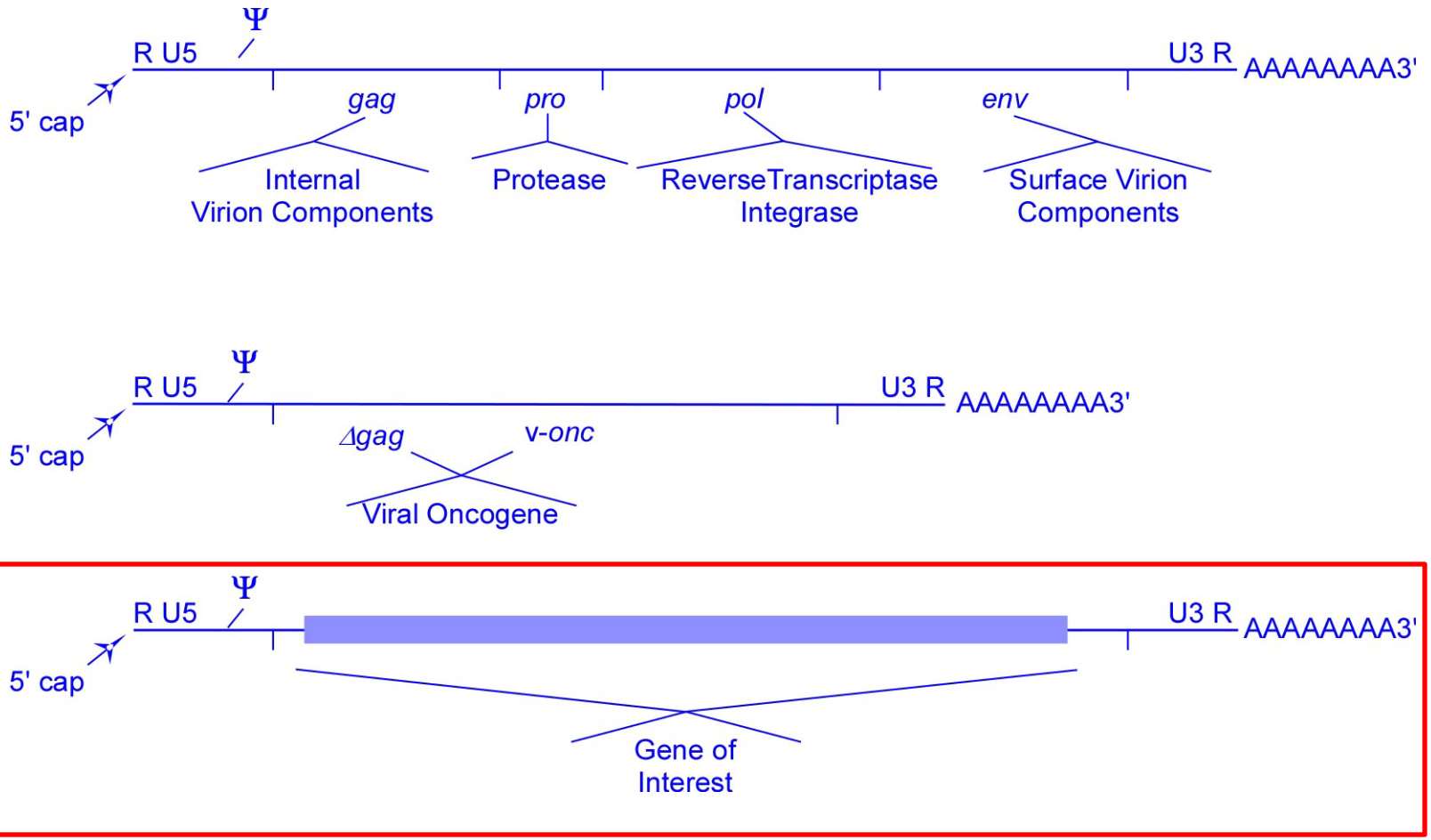
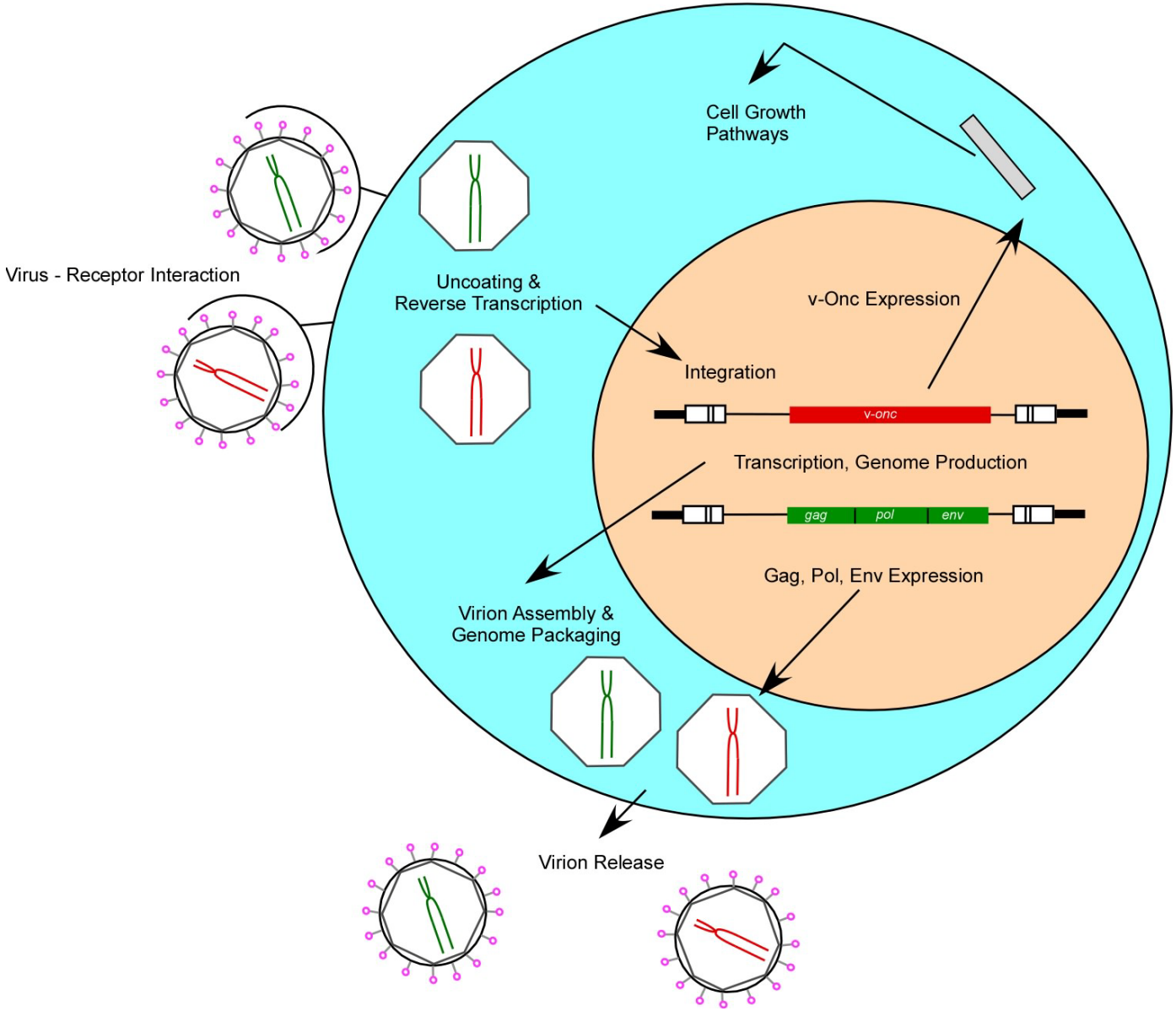
Retroviruses Integrate and Associate with Infected Cells Forever



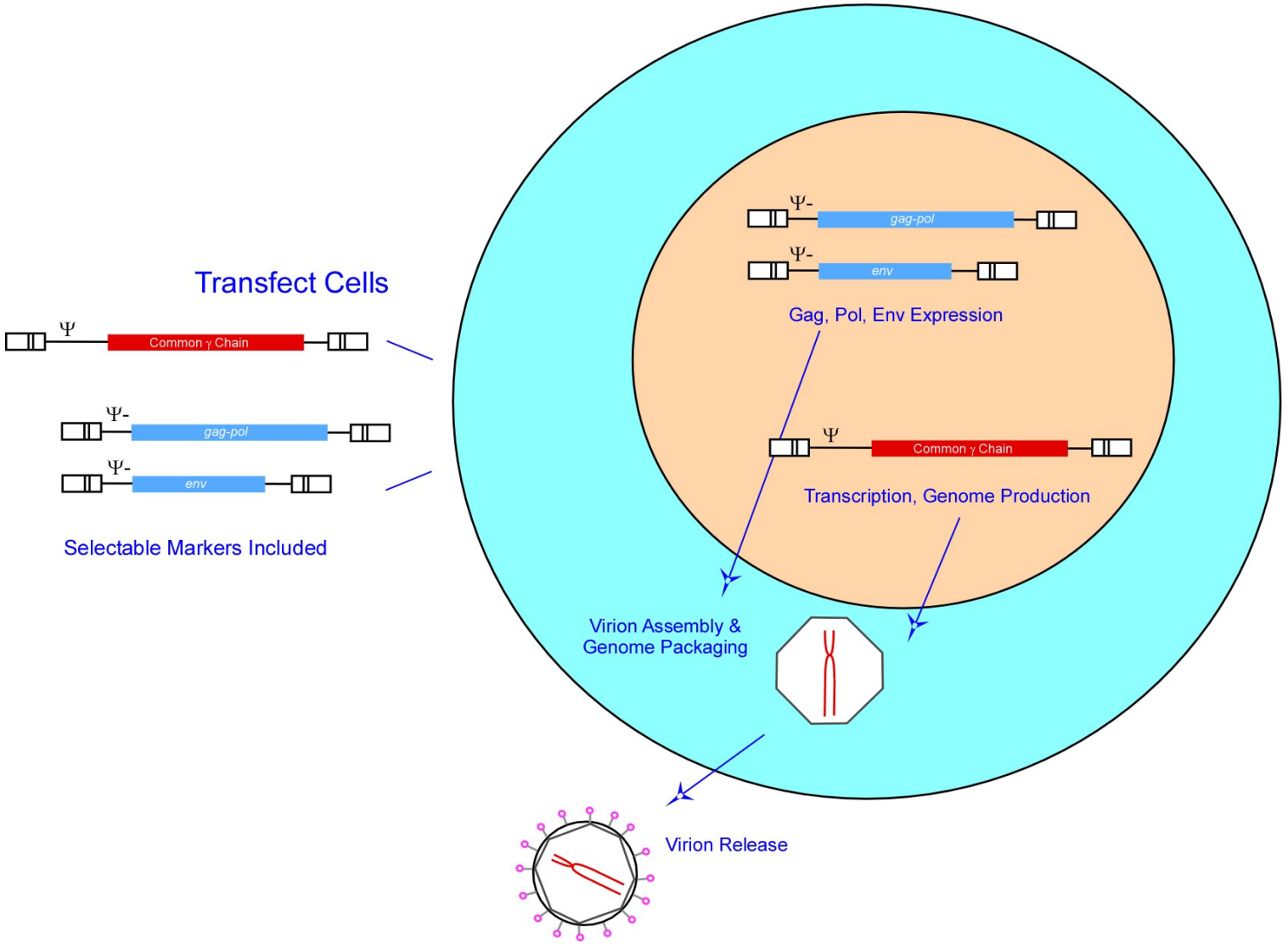
Retrovirus Genome Structure & Vector Development



"Helper" Viruses Enable Replication of Most v-*onc* Gene Containing Retroviruses



Production of Non-Replicating Vector Stocks



Infectious Virions Contain Only the Retrovirus Vector Sequence

Inherent Advantages of Retrovirus Vectors

Biology is well-understood

Reasonable space for “payload” gene

Reliable, relatively high expression

Well controlled approaches to eliminate replicating virus

Reasonable control of multiplicity of infection

Life-long association and production of “payload” gene
(but no rescue strategy)

Use of Retrovirus Vectors for Gene Therapy

Began just over 20 years ago

Gammaretroviruses used initially

Increased use of lentivirus vectors

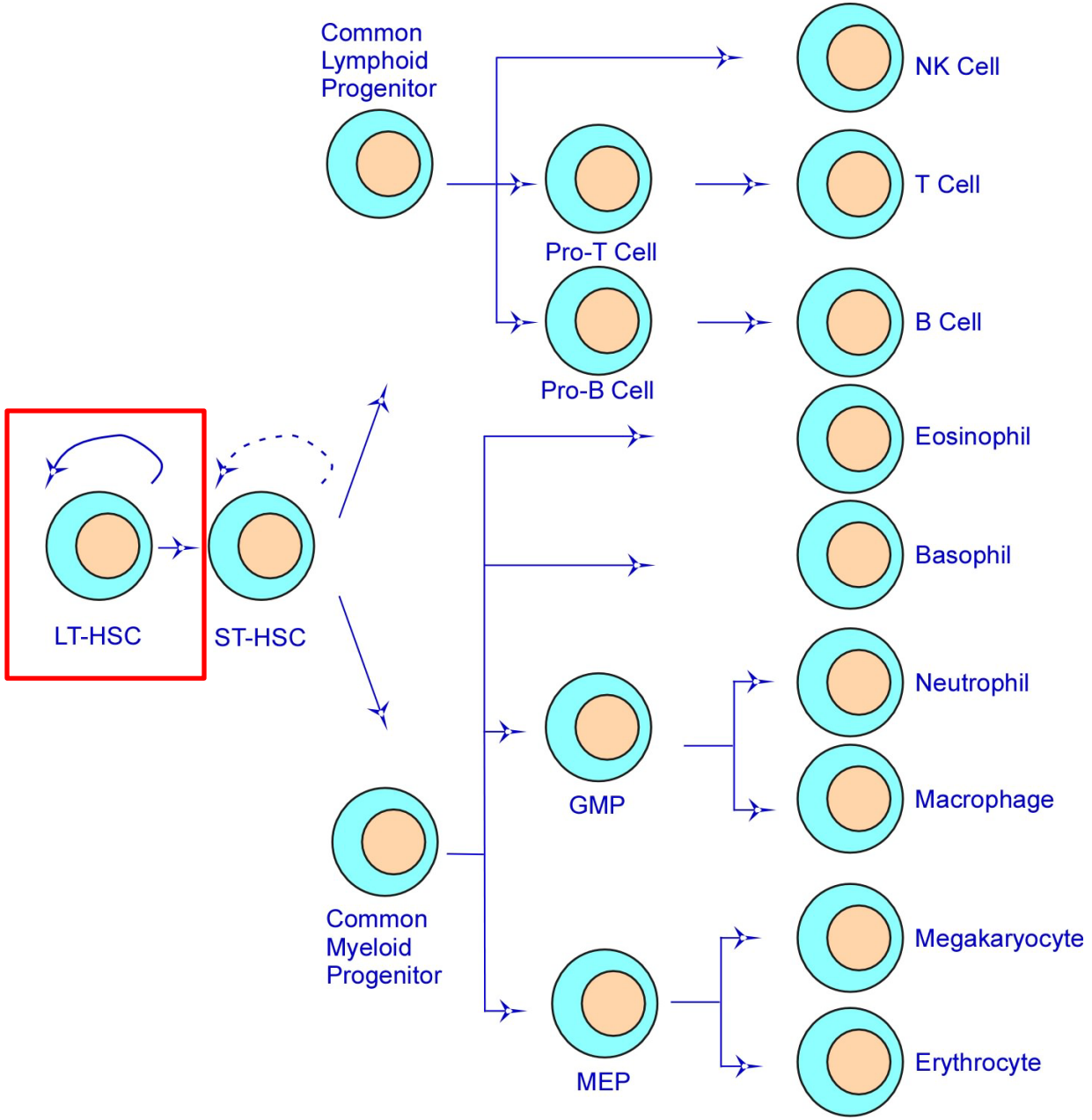
Collectively they have been used in > 600 trials

About 30% of all gene therapy trials in US

Only about 20% focus on congenital diseases

Diseases affecting hematopoietic cells have been a major focus

Hematopoietic Stem Cells – The Vector Target Cells



Overview of Today's Presentation

- Retrovirus Vectors
- SCID-X1 Gene Therapy
- New Approaches – New Questions

Gene Therapy for SCID-X1 – The First Success

Gene Therapy of Human Severe Combined Immunodeficiency (SCID)–X1 Disease

Marina Cavazzana-Calvo,*[1,2,3] Salima Hacein-Bey,*[1,2,3] Geneviève de Saint Basile,[1] Fabian Gross,[2] Eric Yvon,[3] Patrick Nusbaum,[2] Françoise Selz,[1] Christophe Hue,[1,2] Stéphanie Certain,[1] Jean-Laurent Casanova,[1,4] Philippe Bousso,[5] Françoise Le Deist,[1] Alain Fischer[1,2,4]†

Science 288: 669, 2000

Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector

H Bobby Gaspar, Kathryn L Parsley, Steven Howe, Doug King, Kimberly C Gilmour, Joanna Sinclair, Gaby Brouns, Manfred Schmidt, Christof Von Kalle, Torben Barington, Marianne A Jakobsen, Hans O Christensen, Abdulaziz Al Ghonaium, Harry N White, John L Smith, Roland J Levinsky, Robin R Ali, Christine Kinnon, Adrian J Thrasher

Lancet 364: 2181, 2004

Parents' joy as 'bubble boy' saved

By John von Radowitz
Apr 3 2002

SCID-X1 is a Severe Immunodeficiency Disease

SCID-X1 is caused by a deficiency in the *IL2RG*

The *IL2RG* gene encodes γc

The disorder is X-linked

IL2RG is located on Xq13.1

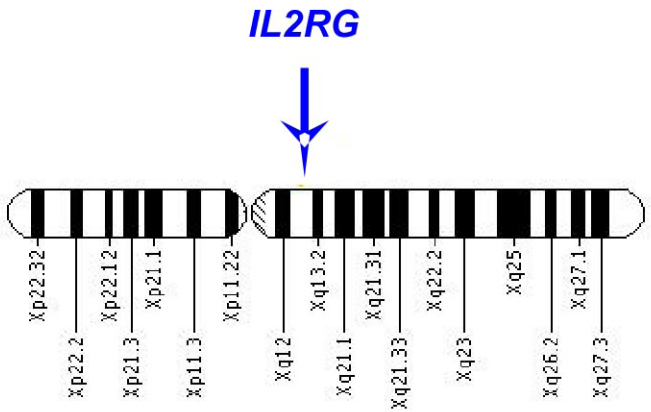
Most loss of function mutations are point mutations

Defects in T cells and NK cells and in B cell responses

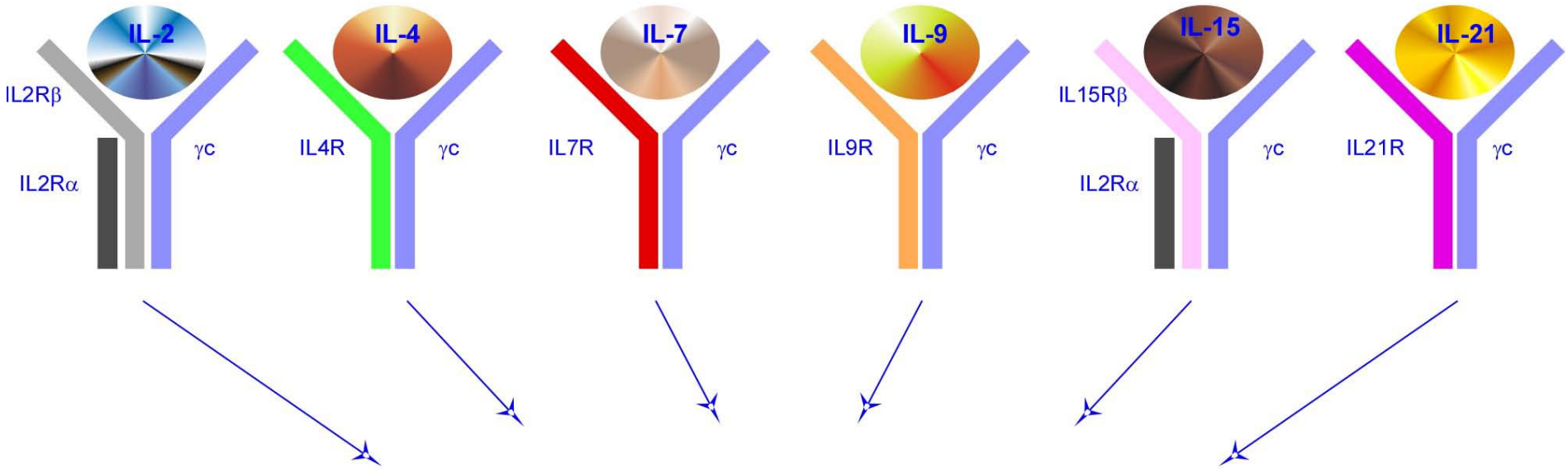
Diagnosis typically made between age 3 and 6 months

Incidence ~ 1 in 100,000 - 500,000 births

About 50% of SCID is SCID-X1



Cytokine Receptors Critical for Hematopoeitic Cells Require the Common γ Chain



JAK-STAT
Downstream Signals Critical for Replication & Differentiation

T cells, NK cells & B cells are especially dependent on these signals.

SCID-X1 Facts

Patients with SCID-X1 are highly susceptible to infection.

Infections are recurrent and begin at 2 – 4 months of age.

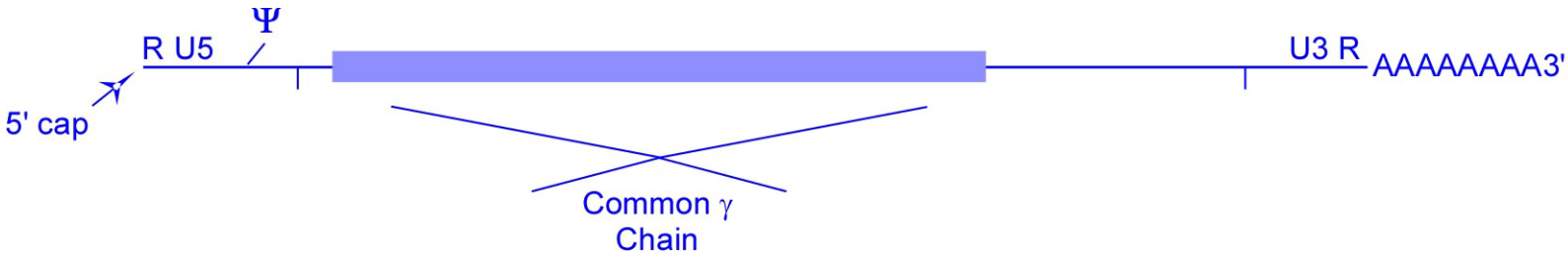
In the absence of treatment, all succumb early in life.

Bone marrow transplantation is a treatment option, but outcome is best with an HLA-matched donor.

In most cases, only a haplo-identical donor is available.

Outcome of haplo-identical transplants can be poor.

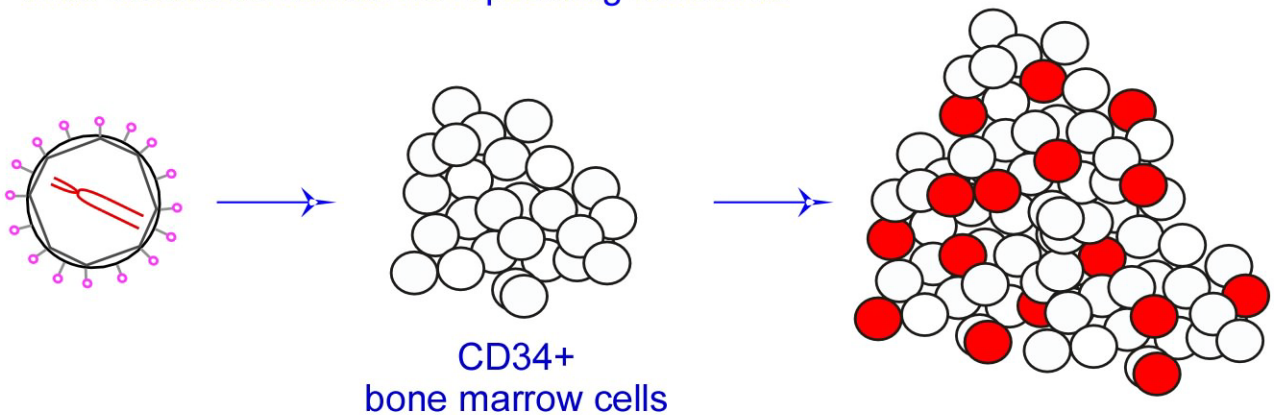
Strategy Used in the SCID-X1 Trials



Common *IL2RG* inserted into a murine retrovirus vector was used

Two different *env* genes used to package the virus

Virus stocks contained no replicating retrovirus



Infuse into patient and monitor reconstitution

Study Subjects in the SCID-X1 Trials

French Trial – Alain Fischer

12 Study Subjects

9 Engrafted

Infants & Very Young Children

British Trial – Adrian Thrasher

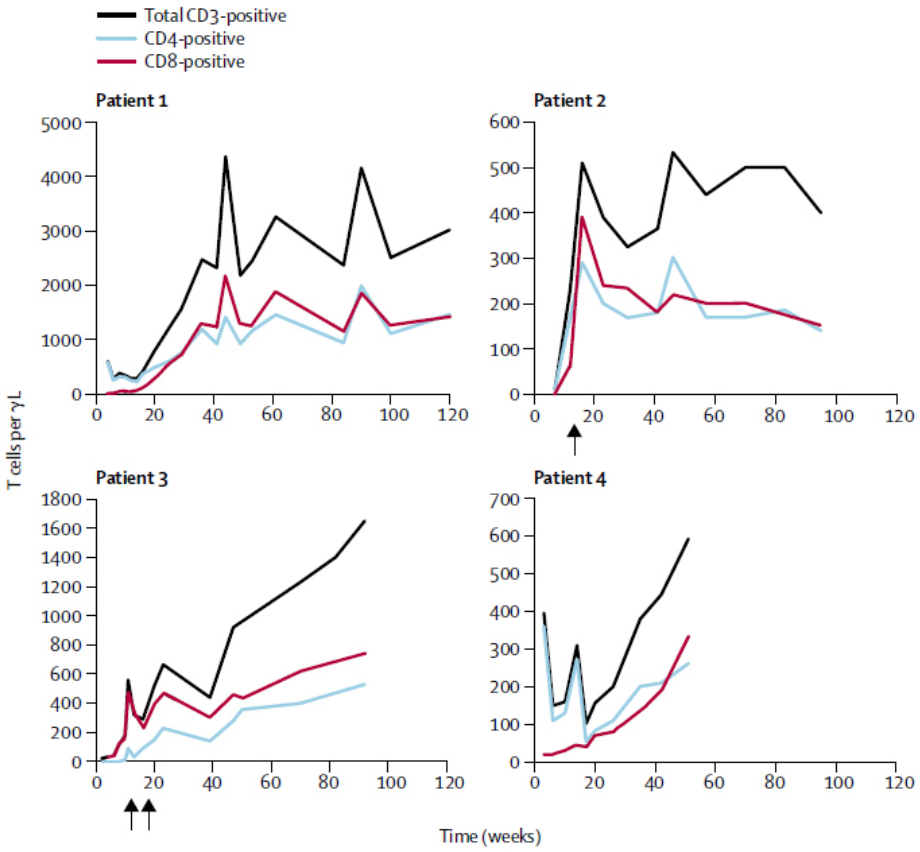
10 Study Subjects

10 Engrafted

Infants & Very Young Children

All Engrafted Study Subjects Showed Lasting Immune Reconstitution

Stem Cell Therapy Restores Immune Function



Other measures of immune function show restoration

B cell responses

NK cell numbers

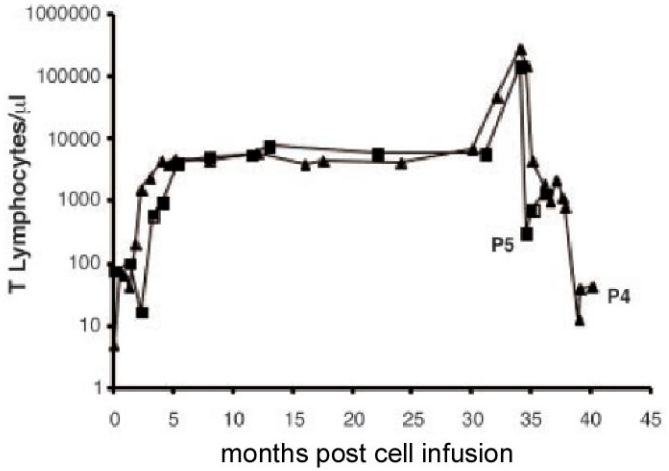
Gasper, et al., Lancet 364: 281, 2004

Leukemias Develop in Some Study Subjects

LMO2-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1

S. Hacein-Bey-Abina,[1,2*] C. Von Kalle,[6,7,8] M. Schmidt,[6,7] M. P. McCormack,[9] N. Wulffraat,[10] P. Leboulch,[11] A. Lim,[12] C. S. Osborne,[13] R. Pawliuk,[11] E. Morillon,[2] R. Sorensen,[19] A. Forster,[9] P. Fraser,[13] J. I. Cohen,[15] G. de Saint Basile,[1] I. Alexander,[16] U. Wintergerst,[17] T. Frebourg,[18] A. Aurias,[19] D. Stoppa-Lyonnet,[20] S. Romana,[3] I. Radford-Weiss,[3] F. Gross,[2] F. Valensi,[4] E. Delabesse,[4] E. Macintyre,[4] F. Sigaux,[20] J. Soulier,[21] L. E. Leiva,[14] M. Wissler,[6,7] C. Prinz,[6,7] T. H. Rabbitts,[9] F. Le Deist,[1] A. Fischer,[1,5†‡] M. Cavazzana-Calvo[1,2‡]

Science 302: 415, 2003

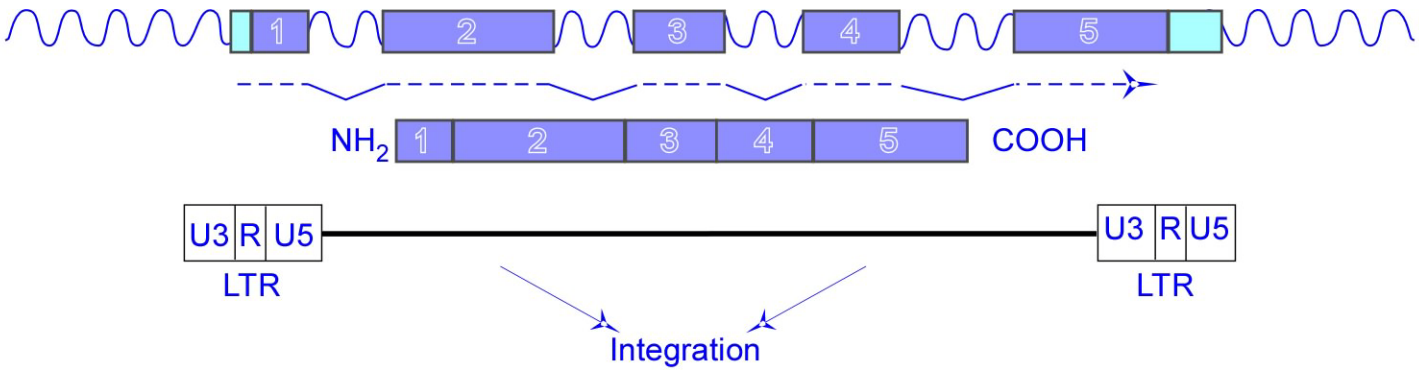


Subsequently, three additional study subjects develop T cell leukemias

Incidence – 4 of 9 in the French trial
1 of 10 in the British trial

Leukemias develop ~30 – 70 months post gene therapy

How Do the Leukemias Develop?



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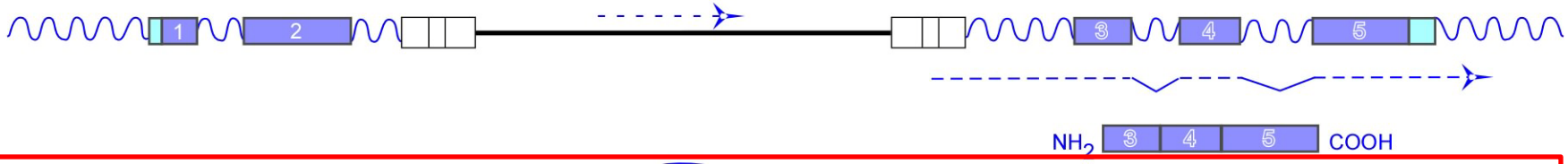

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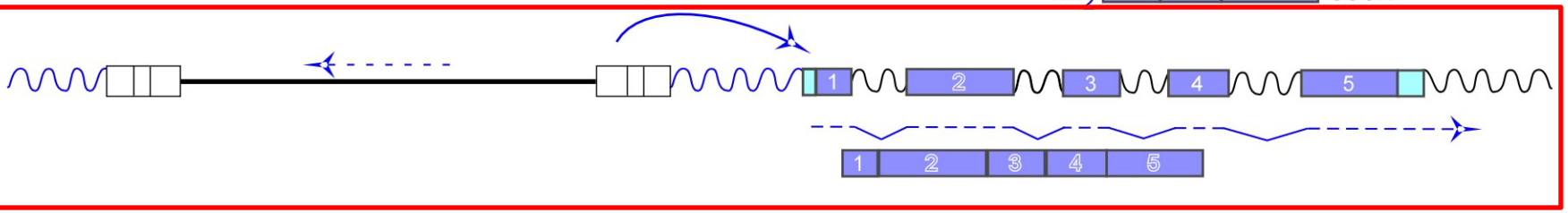

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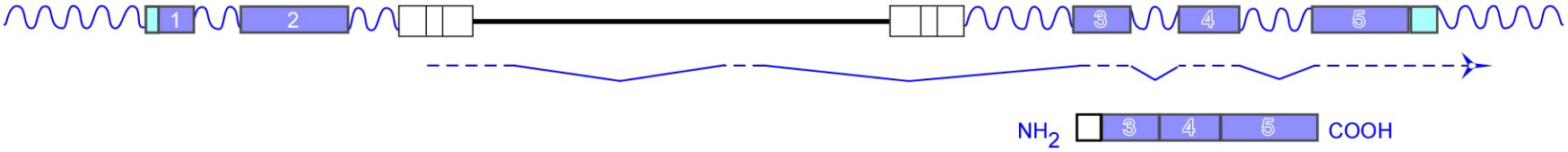
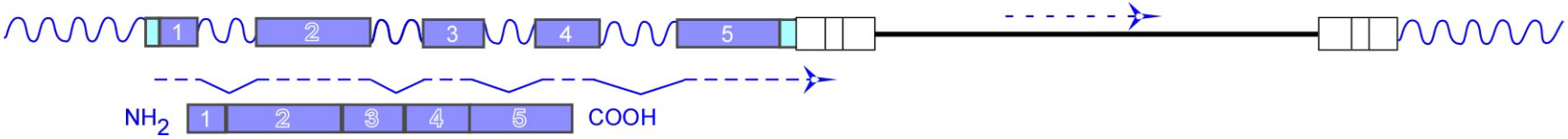
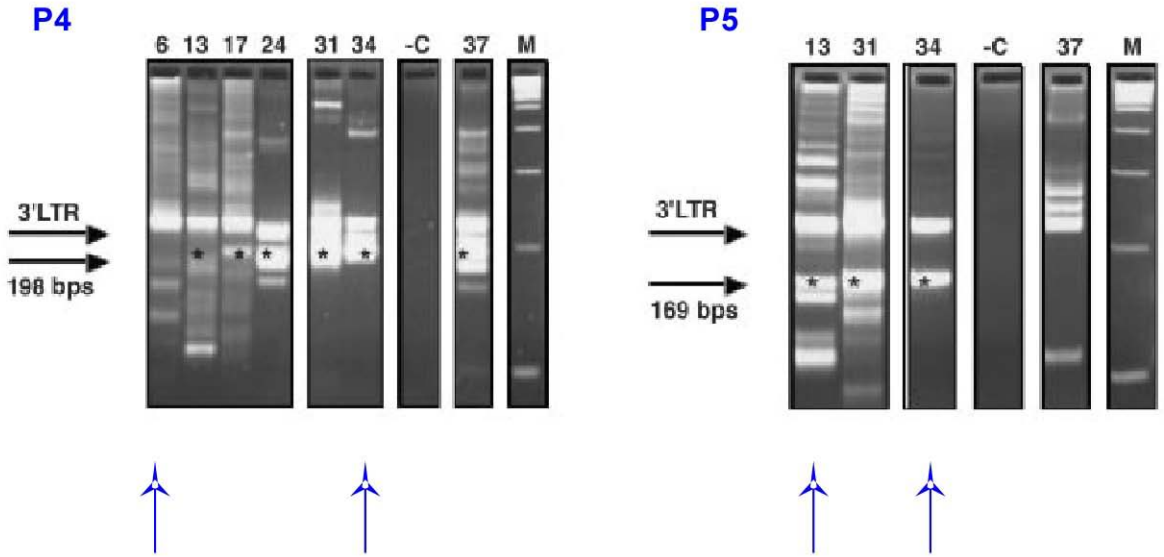


IV.



Common Integration Sites in the Leukemias

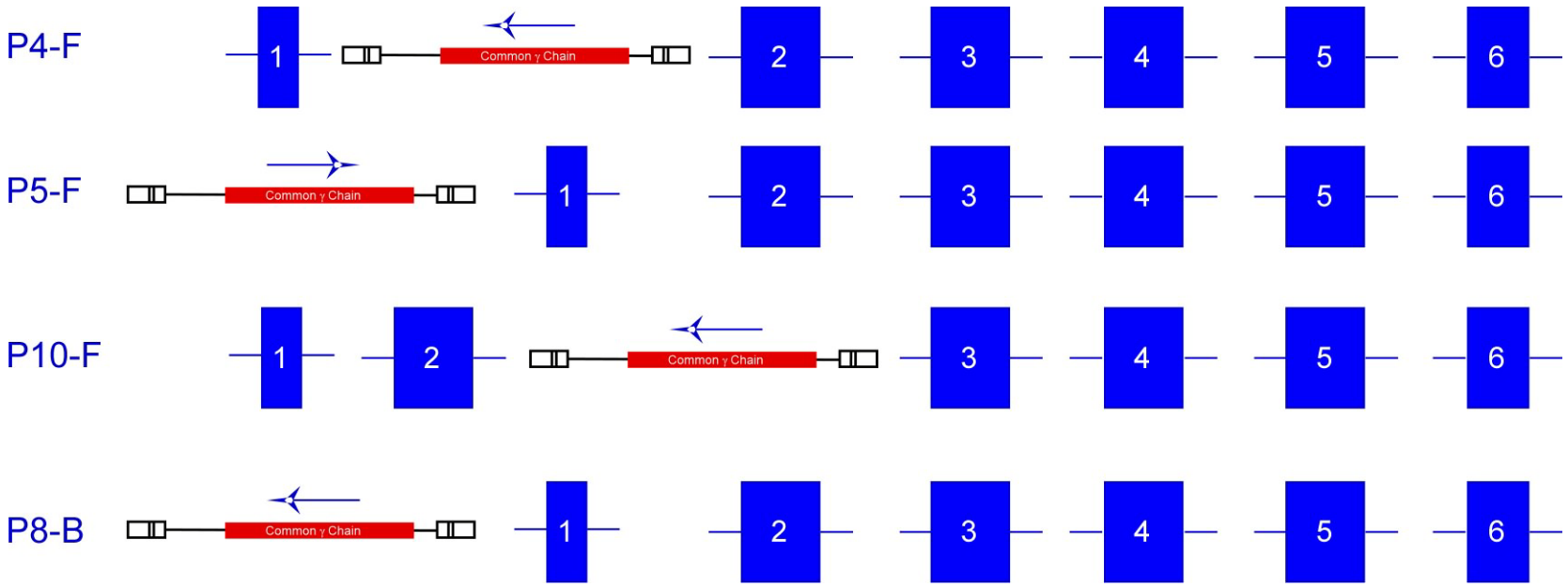
PCR Can be Used to Detect Virus Integrations



Clonal Dominance Emerges in Both Subjects

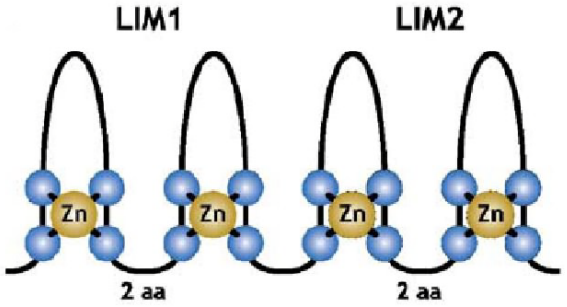
Hacien-Bey-Abina, et al., Science 302: 415, 2003

Integrations in *LMO2* Are Detected in 4 of 5 Subjects



What is LMO2 and How Does it Function?

LMO-2

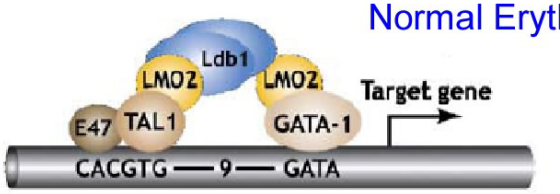


Gene can be activated by chromosomal translocation in T-ALL
Translocation usually involves *Tal/Sil*

Very rare target in murine leukemias and lymphomas induced by murine retroviruses

Normal function involves regulation of early hematopoiesis and vascular remodeling

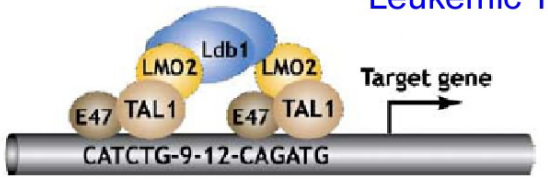
Normal Erythroid Cells



LMO2 functions as part of transcription complexes

LMO2 complexes differ in normal cells and tumor cells

Leukemic T Cells



images from Nam & Rabbits, 2005

Other Changes are Found in the Subjects that Develop Leukemias

P4 – F	*LMO2* integration; t(6,13); *CDKN2A* deletion
P5 – F	*LMO2* integration; *Tal/Sil* translocation; trisomy 10: *NOTCH1* mutation
P7 – F	*CCND2* integration; *CDKN2A* deletion
P10 – F	*LMO2* integration; *BMI1* integration; *NOTCH1* mutation and activation
P8 – B	*LMO2* integration; *NOTCH1* mutation and activation; LOH of *CDKN2A*; *Tal/Sil* translocation

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Strategies to Develop Safer Vectors

The high incidence of leukemia in the study subjects stimulated the gene therapy community to consider strategies to develop safer vectors;

The search for more predictive pre-clinical models was intensified;

Vectors in which expression of the payload gene is not dependent on the LTR were developed;

Lentivirus-based (especially HIV) vectors were already under development and coming into more common use;

Virus expressing these vectors can infect non-dividing cells more readily;

The integration pattern of lentiviruses may decrease the chance of insertional activation.

Integration Preferences Vary for Different Retroviruses

Genome Feature	Human Genome	HIV	MLV
Within 5 kb of start site	~5%	6.9%	26.1%
Within 1 kb of CpG island	~1%	0.2%	11.8%
Within genes	~39%	77.9%	44.3%
Within 1 kb of DNase hypersensitivity site	~1%	~1%	11.4%

Lewinski, et al., PLoS Pathogens 2: e60, 2006

β-Thalassemia Gene Therapy Trial and Lentivirus Vectors

β-thalassemia is a common hemaglobinopathy

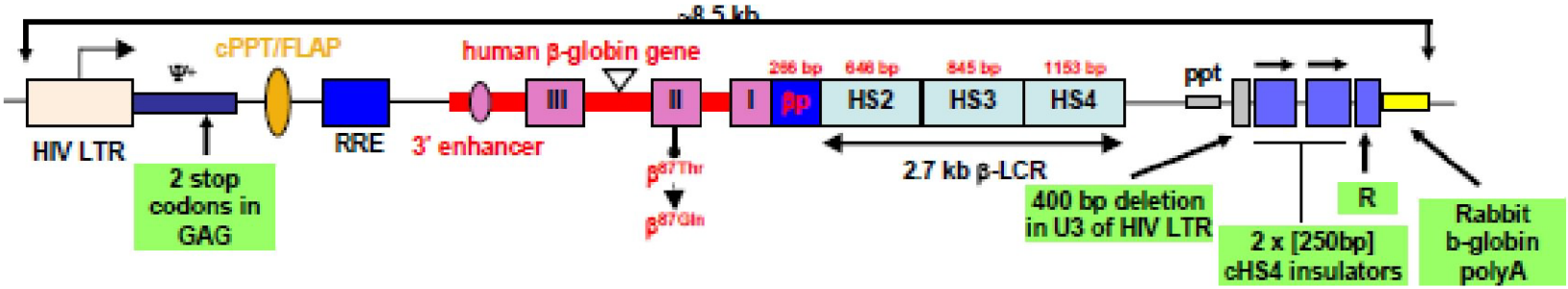
$\beta^E\beta^0$ is a severe form that results in absence of β–globin

Patients are transfusion dependent and develop severe disease as a consequence of the continual transfusions

Hematopoietic stem cell transplantation is the only curative therapy

Life expectancy is reduced and quality of life is highly impaired

Experience with New Vectors - β-Thalassemia



Salient Features of the Vector

HIV LTR with deletion in right LTR

Ψ + with 2 stop codons in *gag*

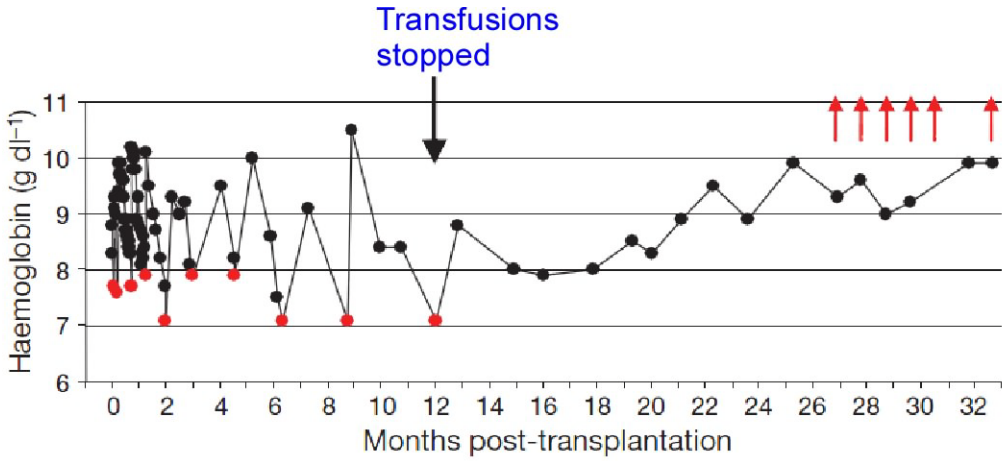
Locus Control Region

cHS4 Insulators (one lost in ~33% transduced cells)

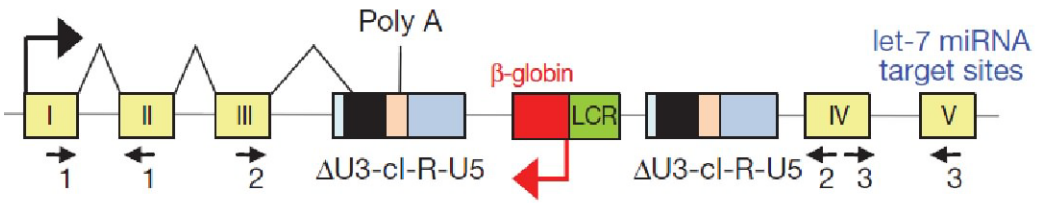
Internal polyA site

Cavazzana-Calvo, et al., Nature 467: 318, 2010

Analyses of the First Study Subject



Increased hemoglobin levels in the absence of transfusion



Cavazanna-Calvo, et al., Nature 367: 318, 2010

Dominant clone with an integration into the *HMGA2* gene

Truncated transcripts

Truncated protein

Study subject remains healthy with no evidence of malignancy

Can the Challenges Presented by Gene Therapy Be Overcome?

Can retrovirus vectors be made that avoid the problems of insertional mutagenesis?

What sorts of model systems can insure that vectors are safe?

Is a 50% incidence of leukemia for SCID-X1 patients “acceptable” until new approaches are available?

How should appropriate ethical standards be applied when study subjects are children?

What standards should regulatory agencies apply in considering approval of these types of trials?

Is the current experience with gene therapy really different from the problems associated with the development of other therapies such as chemotherapy and radiotherapy?